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Review

Splicing factor mutations and cancer

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ABSTRACT

Recent advance in high-throughput sequencing technologies unexpectedly revealed that somatic mutations of splicing factor genes frequently occurred in several types of hematological malignancies including myelodysplastic syndromes (MDS) and other myeloid neoplasms and chronic lymphocytic leukemia (CLL). Spicing factor mutations have been also reported in solid cancers such as breast cancer, pancreatic cancer, uveal melanoma and lung adenocarcinoma. These mutations were heterozygous and mainly affected *U2AF1* (*U2AF35*), *SRSF2* (*SC35*), *SF3B1* (*SF3B155* or *SAP155*) and *ZRSR2* (*URP*), which are engaged in the initial steps of RNA splicing including 3' splice site recognition, and occurred in a largely mutually exclusive pattern, suggesting a common impact of these mutations on RNA splicing. Here we describe splicing factor mutations reported in various types of cancers, their functional/biological effect and their potential as therapeutic targets.

Keywords:
Splicing factor, mutation, cancer

INTRODUCTION

RNA splicing is the process of generating a mature mRNA (messenger RNA) from pre-mRNA during which exon-intron borders are recognized and the intervening intronic sequences are removed.[1] RNA splicing enables production of multiple different mRNA species by alternative splicing of exons or exon segments, which are found in nearly 95% of mammalian genes to generate tissue- and species- specific differentiation patterns.[2] RNA splicing is catalyzed by the spliceosome, which is composed of five small nuclear ribonucloproteins (snRNPs) and a large number of auxiliary proteins.[1] On the other hand, there are two types of introns, termed U2 and U12 type; U2-type introns account for more than 99.5% of introns, whereas U12-type introns constitute less than 0.5% of introns and reside in 700-800 genes. These introns are spliced by the U2-type and U12-type dependent spliceosomes, which are referred to as ‘major’ and ‘minor’ spliceosomes, respectively.[3] The U1, U2, U4/U6, U5 snRNPs are the main components of the major spliceosomes and each snRNP consists of a small nuclear RNA (snRNA) and a varying number of associated proteins. Catalytic process of pre-mRNA splicing is a two-step process, during which splicesomes are assembled in a stepwise fashion as splicing proceeds.[1, 2] In the first step, the 5' splice site is cleaved and, in a coordinated reaction, the 5' G residue of the intron is linked to an A residue near the 3' splice junction. In the second step, the 3' splice site is cleaved, producing ligated exons and excised "lariat" introns **(Figure 1)**.

Recently, several lines of evidence suggest that RNA splicing is also associated with transcription and splicing factors also play an important role in the transcriptional regulation[2], although transcription from DNA to pre-mRNA and pre-mRNA splicing were previously considered as separated processes. For example, splicing factor SC35 (SRSF2) has been shown to affect transcriptional elongation in a gene specific manner.[4] In addition, close interactions between the alternative splicing mechanism and chromatin modifications also has been suggested.[2]

One of the recent, rather unexpected, findings in cancer genetics using high-throughput sequencing is frequent mutations of splicing factor genes found in myeloid dysplasias and to a lesser extent, in other hematopoietic neoplasms and solid cancers.[5-7] Among major targets of these mutations are *U2AF1* (*U2AF35*), *SRSF2* (*SC35*), *SF3B1* (*SF3B155* or *SAP155*) and *ZRSR2* (*URP*), which are known to be commonly involved in in the initial steps of RNA splicing. This review summarizes the splicing factor mutations in various types of cancers and discusses the mechanisms by which these mutations lead to carcinogenesis and their potential as therapeutic targets.

RNA splicing and disease

Alterations in RNA splicing have been implicated in various types of human diseases through disruption of either *cis*-acting elements within affected genes or *trans*-acting factors that are required for normal splicing or splicing regulation.[8] In general, disruption of *cis*-acting elements affects only a single gene, whereas defects in *trans*-acting factors could impact on multiple genes. Disruption of *cis*-acting elements is a well-known mechanism causing abnormal translation of protein; as many as 50% of disease-causing mutations are estimated to be *cis*-acting mutations that affect RNA splicing, leading to altered protein translation.[9] Other alterations of *trans*-acting elements, in which germline mutations affect major spliceosome components, including four components of U4/U5/U6 tri-snRNP complex, *PRPF31*, *PRPF8*, *PRPF3* and *RP9*, have been recognized as the causes of autosomal dominant forms of retinitis pigmentosa.[10] Germline mutations of *SF3B4*, a component of U2 snRNP, have been shown to be the cause of Nager syndrome.[11] More recently, mutation of the minor spliceosome component, RNU4ATAC, which codes U4atac snRNA, is also reported as a cause of MOPD I (Taybi-Linder syndrome).[12, 13] Therefore mutations in ubiquitous spliceosome components actually cause only tissue-specific phenotypes. This cell type specificity is likely to result from sensitivitiy of some cell-specific pre-mRNAs to dysfunction of each spliceosome component.[8, 10] For example, pre-mRNA of *RDS* and *FSCN2*, whose expression are specific to photoreceptor, were shown to be sensitive to *PRPF31* mutations.[14]

RNA splicing and cancer

Deregulated or abnormal RNA splicing has been considered as a common finding in cancer cells and causally related to carcinogenesis[15], although a more recent analysis revealed that the prevalence of alternatively spliced genes was actually slightly lower in tumors compared to normal tissues.[16] For example, a variety of cancer cell types show increased expression of the longer, anti-apoptotic splicing form of the Bcl-x gene, while another shorter isoform (Bcl-x(s)) promotes apoptosis.[15],[17] Caspase-2 also generates both proapoptotic (caspase-2L) and anti-apoptotic isoforms (caspase-2S) by alternative splicing.[18] Similarly, short isoform of Fas (sFas) also have been shown to be antiapoptotic and elevated production of that has been observed in various types of cancers.[19] Although the molecular mechanism of these abnormal splicing is largely unknown, a recent report suggests a possible role of deregulated *trans*-acting factor. A member of the SR protein family, SRSF1 (SF2/ASF), is involved in RNA splicing and highly expressed in various types of human cancers, where an oncogenic role of the protein was indicated by transformation of SRSF1-overexpressed fibroblasts, although no oncogenic *SRSF1* mutation has been thus far reported.[20] SRSF1 is also shown to control alternative splicing of tumor

suppressor BIN1, MNK1 and S6K1 kinases, which could contribute to the oncogenic action of SRSF1. However, more direct evidence suggesting oncogenic role of *trans*-acting splicing factors has been obtained through comprehensive analysis of somatic mutations in a variety of cancer types using massively parallel sequencing, in which multiple components of the RNA splicing machinery were shown to be recurrently mutated.

Splicing factor mutations in hematological malignancies

MDS and related disorders are a heterogeneous group of myeloid neoplasms characterized by varying degrees of cytopenia and transition to acute myeloid leukemia (AML).[21] Recently, several groups identified frequent somatic mutations of the RNA splicing machinery in MDS using whole exome/genome sequencing.[22-25] Mutations are heterozygous and highly frequent (45%-85%) in and specific to MDS and related myeloid neoplasms with myelodysplastic features, but less common (10%) in patients with de novo AML[22, 26] and myeloproliferative neoplasms (MPN) [22, 27] (Table 1) and rarely found in pediatric myeloid malignancies[28, 29] and lymphoid neoplasms[30] except for chronic lymphocytic leukemia (CLL).[31-33] Although more than 30 splicing factors are known to be mutated in at least one patient[22, 26, 32, 34, 35] (Table 1), 4 genes represent the major mutational targets, including *U2AF1*, *SRSF2*, *SF3B1* and *ZRSR2.* Most of the mutations affected the components that are involved in the initial steps of pre-mRNA splicing including 3'-splice site recognition and occurred in a mutually exclusive manner, suggesting a common impact of these mutations on RNA splicing in the pathogenesis of myelodysplasia **(Figure 2)**. Another conspicuous feature of spliceosome mutations is the presence of mutational hot spots in *U2AF1, SRSF2* and *SF3B1* and the lack of nonsense/frameshift changes, indicating that functional consequence of these mutations should not be a simple loss-of-function, but accompanies some gain-of-function. As mentioned below, however, the frequency of mutations differs significantly across disease types; *SF3B1* mutations are extremely frequent in those MDS phenotypes showing ring sideroblasts compared to other MDS subtypes, while *SRSF2* mutations are more common in chronic myelomonocytic leukemia (CMML) (28-47%). Thus, despite common association of different splicing factor mutations to MDS phenotypes, the functional/biological impact of each mutation may not be completely identical, but probably reflecting discrete function of each spliceosome component, critically different, which might contribute to associated disease phenotypes. *SF3B1* mutations were also reported in 9.7-15% of CLL cases[31-33], in which these mutations tend to represent subclonal mutations and were significantly associated with poor clinical outcome.[36] The functional basis

for the common *SF3B1* mutations in chronic myeloid neoplasms and lymphoid malignancies is still unknown but this may indicate a possibility of the common origin of these malignancies.

Mutated spliceosome components

U2AF1

U2AF1 (U2AF35: 35 kDa subunit of U2AF) is tightly bound to U2AF2 (U2AF65: 65 kDa subunit of U2AF), consisting of the U2 auxilliary factor (U2AF) heterodimer.[1] U2AF1 interacts with the AG dinucleotide of the 3' splice site through its RNA recognition motif (RRM) and also play a role in juxtaposing the 5' and 3' splice sites by binding to SR proteins such as SRSF2 through its arginine-serine-rich (RS) domain. In addition, the pre-mRNA branch site and polypyrimidine tract (Py-tract) are bound by SF1/BBP and U2AF2, respectively and these interactions yield the spliceosomal E complex and play crucial roles in the recognition of the 3' splice site of an intron. *U2AF1* is mutated in 5-16% of MDS and 5-17% of CMML. More recently, recurrent *U2AF1* mutations have been reported in hairy-cell leukemia-variant (HCVv).[37] Most of reported *U2AF1* mutations were observed at two amino acid positions, S34 and Q157, which are located within evolutionally conserved zinc finger domains **(Figure 3)**.[22]

SRSF2

SRSF2 (SC35) is a member of the SR protein family of proteins and thought to interact with exonic splicing enhancers (ESEs) thorough its RRM domain and recruits U2AF to the upstream 3' splice site and U1 snRNP to the downstream 5' splice site, giving positive effects on splice-site usage.[38] *SRSF2* mutations were more frequent in CMML (28-47%). Most of reported mutations or insertion-deletions of *SRSF2* exclusively involved P95 within the intervening sequence between the RRM and RS domains, indicating the gain-of-function nature of these mutations **(Figure 3).**[22] *SRSF2* mutations are also reported in other myeloid malignancies including MPN transformed to secondary AML[39] and systemic mastocytosis.[40]

SF3B1 (*SF3b155*)

SF3B1 is a component of the SF3B complex, which consists of U2 snRNP together with the SF3A complex. U2 snRNP bind to a branch point sequence (BPS) via SF3B14 (p14) and also to U2AF2 via SF3B1 (SF3b155) to stabilize the interaction between the U2 snRNA and pre-mRNA's BPS, leading to the establishment of the spliceosomal A complex.[1] Although *SF3B1* mutations are found in a variety of myeloid malignancies, extremely high mutation rates have been reported in those MDS subtypes showing increased ring sideroblasts (>15% of all erythroblasts in bone marrow), including refractory anemia with ring sideroblasts (RARS) (64-83%) and refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS) (37-76%).[22, 25, 41, 42]

SF3B1 mutations were also common in RARS associated with marked thrombocytosis (RARS-T) (67-85%)[25, 41, 43], which has been classified as a unique form of myelodysplastic/myeloproliferative neoplasms (MDS/MPN) showing increased ring sideroblasts and an elevated platelet count. Moreover, even in other MDS subtypes, *SF3B1* mutations were associated with significant increased, although less than 15%, ring sideroblasts, in which the presence of *SF3B1* mutations showed very high positive and negative predictive values (~97.7% and 98.7%, respectively) for the presence of ring sideroblasts.[41] *SF3B1* mutations clustered in several hot spots, such as K700, E622, R625, H662 and K666, located in HEAT domains that extend through exon12 to 15 **(Figure 3)**.[22, 23]

***ZRSR* (*URP*)**

ZRSR2 functions at 3' splice site of both U2- and U12-type introns[44] and participates in the formation of the splicing A complex on 3' splice sites recognition. In addition, ZRSR2 is also engaged in the second step of splicing at U2-type intron.[44] *ZRSR2* mutations were widely distributed along the entire coding region and most of them were nonsense mutations or frameshift changes, or involved splicing donor/acceptor sites that caused either a premature truncation or a large structural change of the protein, leading to loss-of-function **(Figure 3)**.[22] *ZRSR2* mutations are less frequent in MDS compared to mutations of *U2AF1*, *SRSF2*, and *SF3B1*, and were associated with refractory anemia with excess blast (RAEB) and the international prognostic scoring system (IPSS) int-1 or int-2 risk-groups.[45]

Other splicing factors

Other splicing factors have been also reported to be recurrently mutated in myeloid malignancies, although low frequencies of these mutations make their role in myeloid leukemogenesis being less established. These include an additional two components of splicing A complex (SF3A1 and U2AF2), PRPF40B, whose functions in RNA splicing is poorly defined[22], LUC7L2, a homologue of the putative RNA-binding protein Luc7, PRPF8 and other factors.[26, 32] LUC7L2 is thought to be involved in recognition of 5' splice site and its inactivation mutations have been reported in MDS and secondary AML.[35, 46] PRPF8 (pre-mRNA processing factor 8) is a highly conserved component of both major and minor spliceosomes and its mutations also have been reported in de novo and secondary AML. [26, 35] Of note is that a recent study showed that truncating mutation of *prpf8* impaired myeloid differentiation in Zebrafish, suggesting its essential role in hematopoietic development.[47]

Clinical impact of spliceosome mutations

Despite their common role in myeloid leukemogenesis anticipated from their non-overlapped distribution and functional implications, they seemed to have different impacts on clinical outcome in MDS and other myeloid malignancies. It seems that *SF3B1* mutations are consistently associated with better overall survival (OS) and leukemia free survival (LFS) in MDS and RARS-T,[23, 41] although initial reports showed conflicting observations.[42, 48]

SRSF2 mutations are more common in advanced MDS subtypes (RAEB) and associated with a significantly shorter OS and LFS,[35, 49] while these mutations seemed to have no significant impact on prognosis among CMML patients.[50, 51] *SRSF2*-mutated AML transformed from MPN also have a shorter OS.[39] In addition, Lasho et al. and Vannucchi et al. reported *SRSF2* mutations were associated with poor prognosis in primary myelofibrosis (PMF) patients.[52, 53] *U2AF1* mutated MDS cases are associated with faster progression to secondary AML with no significant effects on OS or event free survivals[24],[54], whereas another group reported a significantly shorter OS for *U2AF1* mutated myeloid malignancies.[35] *ZRSR2* mutations did not seem to affect prognosis.[55] The IPSS and the revised IPSS (IPSS-R) have been widely used for prognostication of MDS patients, however, this does not take into account for somatic mutations. Therefore, there are several attempts to integrate the mutational information of these genes into the prognostic model of MDS.[55, 56]

On the other hand, *SF3B1* mutations on CLL were associated with poor prognosis[31, 32, 57] and more advanced fludarabine refractory disease.[33] Rossi et al. has proposed an integrated prognostic model of cytogenetic and molecular markers including *SF3B1* mutations.[58]

Splicing factor mutations in solid cancers

Splicing factor mutations also have been reported in a wide variety of solid cancers, although generally at much lower frequencies. *SF3B1* has been reported to be mutated in less than 4% of breast cancer, endometrial cancer, bladder cancer, pancreatic ductal adenocarcinoma and adenoid cystic carcinoma, where mutations were distributed at the same hot spots as reported in MDS and CLL, such as K700E and K666Q.[23, 59-62] *SF3B1* mutations were also common in uveral melanoma, in which most of the mutations almost exclusively involved R625.[63, 64] In uveal melanoma, the R625 mutations were associated with a lower metastasis rate and good prognosis, whereas those at other nucleotide positions were found in uveal melanomas with disomy 3 that developed metastases.[64] The *U2AF1* S34F mutation was found in 3% of the cases with lung adenocarcinoma.[65]

Splicing factor mutations and disease progression

Deep sequencing of mutant alleles in cancer specimens provides a clue to speculate the origin of splicing factor mutations during clonal evolution.[24],[41] Thus, in the majority of cases with myeloid malignancies analyzed, *SF3B1* and *U2AF1* mutations generally showed a high allelic burden in both CD34-positive cell and granulocyte derived DNA, suggesting that these mutations were acquired during early development of multipotent hematopoietic stem cells and transmitted to their myeloid progenitors.[24],[41] Recent high-throughput targeted sequencing of 94-111 genes in a large cohort of patients with MDS and related neoplasms also confirmed that splicing factor mutations were typically acquired early. [55, 56] Similarly, *SRSF2* mutations, together with *KIT* D816V and *TET2* mutations, also showed a high allelic burden in advanced systemic mastocytosis and could be early events.[40] Analysis of multiple mutations using single-cell-derived colonies provides more direct information about the order of mutations, which clarified that *SRSF2* mutation is thought to be one of the initiating mutations of CMML pathogenesis together with *TET2* mutation according to their higher prevalence.[66]

However, the founder role of the splicing factor mutation could be variable depending on cases. In the initial report in RARS-T, which is characterized by frequent coexisting mutations of *SF3B1,* and *JAK2 and/or MPL*, *SF3B1* mutations showed higher allelic burden compared to coexisting *JAK2/MPL* mutations, suggesting that the *JAK2* or *MPL* mutations occurred in a pre-existing *SF3B1*-mutated cell.[41] In a recent report, in contrast, higher allelic burden of *JAK2* than *SF3B1* mutant alleles indicated that *SF3B1* mutations in a pre-existing MPN clone having a *JAK2* mutation was implicated in the development of RARS-T.[67] Also, in the case of CLL, estimation of multiple driver mutations/chromosomal lesions using whole-exome sequencing and copy number analyses revealed that *SF3B1* mutations represented subclonal lesions in an approximately half of the *SF3B1*-mutated CLL cases.[36] Similarly, *U2AF1* mutations in HCLv were also thought to be subclonal mutations because of their lower allele frequencies.[37]

Functional impacts of splicing factor mutations

Despite strong genetic evidence of their involvement in clonal selection, the molecular mechanism of splicing factor mutations remains largely unknown. When overexpressed in HeLa cells, mutant U2AF1, but not the wild-type allele, induces severe suppression, rather than promotion, of cell proliferation with severe cell cycle arrest at G2/M transition and increased apoptosis.[22] Mutant U2AF1-transduced hematopoietic stem/progenitor cells show compromised repopulating capacity.[22] However, the interpretation of these observations may be complicated by the fact that transduction of oncogenic alleles potentially induces cellular stress that could result in cell

senescence or induction of apoptosis.[68] While the hot-spot mutations always exists in heterozygous state, overexpression of these mutant alleles may rather mimic homozygous mutations in their expression level. Thus more sophisticated evaluation would be required to evaluate the oncogenic function of the splicing factor mutations, possibly using appropriate mouse model. On the other hand, some evidence suggested that other driver mutations or cytogenetic changes could contribute to the clonal dominance of cells with splicing factor mutations cooperatively. For example, in MDS, *SRSF2* mutations frequently co-occurred with *IDH2* [56] and *TET2* mutations.[55] Similarly, in CLL, *SF3B1* mutations were frequently co-occurred with del(11q).[31]

Given the critical role of these splicing factors in pre-mRNA processing, one would speculate that their oncogenic actions are through de-regulated pre-mRNA splicing, leading to abnormal protein expression of relevant genes. In fact, when transduced in HeLa cells, the U2AF1 S34F mutant allele induces a variety of abnormal RNA splicing in a global manner, including exon skipping, unspliced intron, altered splice site recognition and altered exon usage ([22] and unpublished observation) **(Figure 4)**. Abnormal exon skipping in mutant U2AF1-transduced cells was also demonstrated using a minigene reporter assay.[24] Of interest, an increased splicing activity was demonstrated for U2AF1 S34F mutant-transduced cells compared to wild-type U2AF1-transduced cells in a double-reported splicing assay[24], supporting the putative gain of function of this allele. Visconte et al. performed knock-down experiment of *SF3B1* gene on K562 cell line and showed dysfunction of splicing exclusively affected U2-type introns as expected.[69]

Of note is mutations in ubiquitous spliceosome components actually cause specific phenotypes such as myelodysplasia and CLL. In this context, mutated splicesome components are ubiquitous but not necessarily essential for all introns or splice sites. For examples, AG-independent 3’ splice site which has a long stretch of pyrimidines can bind to U2AF2 without U2AF1.[70, 71] Therefore, one or more sensitive pre-mRNA to these mutations may cause such disease phenotypes. However, currently, little is known about the functionally relevant gene targets for abnormal splicing that would be caused by these mutations, if ever it play a major role in the development of MDS and other cancers. Przychodzen et al. analyzed RNA sequencing data of AML patients with and without *U2AF1* mutation and identified U2AF1 mutant-specific splicing patterns, including exon skipping and exon retention in 35 genes, which are involved in functionally important pathways such as cell cycle and RNA processing.[72] Quesada et al. performed exon arrays and RNA sequencing of CLL samples with *SF3B1* mutations and showed impaired RNA splicing activities of genes such as *FOXP1*, whose splicing variants are known to be associated with diffuse large B cell lymphoma (DLBCL).[32] However, the relevance of abnormal splicing of these

genes to leukemogenesis has not been confirmed and needs further investigation in the future. Papaemmanuil et al. preformed expression analyses of CD34+ bone marrow cells derived from MDS patients and identified 94 genes which were down-regulated in *SF3B1* mutated cases.[23] Among these, 7 of 50 most significantly down-regulated genes were related to mitochondrial functions, deregulation of which could be implicated in abnormal iron-metabolism that is a characteristic of RARS. Furney et al. performed RNA sequencing of uveal melanoma samples and revealed that *SF3B1* mutations were associated with differential alternative splicing of *ABCC5*, *UQCC* and the long non-coding RNA (lncRNA) CRNDE.[73]

Finally, abnormal pre-mRNA splicing may not be the only mechanism that explains the oncogenic capacity of these splicing factor mutations. Several lines of evidence indicate close coupling between transcription and pre-mRNA splicing[4], in which possible interactions between epigenetic regulators and the splicing machinery.[74, 75] In addition, close associations between mutation types and disease subtypes could be better explained by the discrete function of each mutation other than the common function in pre-mRNA splicing. For example, sf3b1 is localized on the 5' region of the Hox gene cluster and physically interacts with different components in polycomb repressive complex 1 (PRC1), such as Zfp144 and Rnf2, which seem to be functionally relevant interactions. In fact, *sf3b1*+/− mice showed posterior transformation of vertebrae with abnormal Hox gene expression but not with abnormal splicing of the relevant Hox gene transcripts, which is repressed by the background of trithorax mutation (mll mutation).[76] It has been demonstrated that SRSF2 was also involved in the regulation of DNA stability and that depletion of SRSF2 can lead to genomic instability.[77] Of interest in this context, regardless of disease subtypes, samples with *SRSF2* mutations were reported to have significantly more mutations of other genes compared with *U2AF1* mutations.[22]

Therapeutic implication of splicing factor mutations

Given the essential role of pre-mRNA splicing in cell homeostasis, inhibition of splicing factors using low molecular weight compounds that targets mutated spliceosome components could be an interest of possible therapeutics, because cancer cells carrying these splicing factor mutations are likely to have compromised pre-mRNA splicing and could be more sensitive to these compounds than unmutated cells (synthetic lethality) **(Figure 5)**.[78] Homozygous deletion of sf3b1 allele results in lethality in the very early step during development.[76] Several compounds have been known to inhibit pre-mRNA splicing. Spliceostatin A is the methylketal derivative of natural product FR901464, who has anti-cancer activity, and inhibit RNA splicing through the connection with SF3B complex.[79] E7107 is the derivative of natural product pladienolide B with

anticancer effect and also inhibit RNA splicing through the binding with SAP130 (SF3B3).[80] In addition, Sudemycin,[81] which is the analog of FR901464, and Meayamycin,[82] that is totally synthesized FR901464 analog, are also shown to inhibit RNA splicing and show anticancer activity. Although currently, there is no proof of concept for this therapeutic possibility shown experimentally, it would be an intriguing possibility to be tested.

CONCLUDING REMARKS

Splicing factors represent a novel class of mutational targets in cancer, especially in MDS and other hematopoietic malignances, which were unexpectedly revealed by massively parallel sequencing of cancer genome. Most of these mutations involved the spliceosome components involved in the 3' splice site recognition during early steps of RNA splicing, including *SF3B1*, *U2AF1*, *SRSF2* and *ZRSR2*, indicating their oncogenic actions through de-regulated RNA splicing, although the exact mechanism of their oncogenic actions is largely unknown. A potential role of spliceosome inhibition in novel therapeutics for these intractable neoplasms is also an interest of the investigation in the future.

Table 1 Splicing factor mutations identified in various types of cancers

Gene	Disease type	Frequency	Citation
SF3B1	MDS, CMML, AML	82.6%(RARS), 76.0%(RCMD-RS), 6.5%(MDS without RS), 4.5%(CMML), 4.8%(AML/MDS), 2.6%(AML)	Yoshida K, et al. Nature, 2011[22]
	MDS(RARS, RCMD-RS)	20%(MDS), 65%(RARS, RCMD-RS)	Papaemmanuil E, et al. NEJM, 2011[23]
	MDS(RARS, RCMD-RS), MDS/MPN	64%(RARS), 72%(RARS-T)	Visconte V, et al. Leukemia, 2011[25]
	MDS, MDS/MPN	28.1%(MDS), 79%(RARS), 57.7%(RCMD-RS), 66.7%(RARS-T)	Malcovati L, et al. Blood, 2011[41]
	MDS	73%(RARS), 37%(RCMD-RS), 18%(RAEB1-RS)	Patnaik MM, et al. Blood, 2011[42]
	MDS	14.8%	Damm F, et al. Leukemia, 2011[48]
	RARS, RCMD-RS	60.0%	Makishima H, et al. Blood, 2012[35]
	MDS	16.4%	Damm F, et al. Blood, 2012[45]
	Lower-risk MDS	22.0%	Bejar R, et al. JCO, 2012[83]
	MDS, secondary AML	7.0%	Walter MJ, et al. Leukemia, 2013[84]
	MDS	16.0%	Mian SA, et al. Haematologica, 2013[85]
	MDS, MDS/MPN	24%	Papaemmanuil E, et al. Blood, 2013[56]
	MDS, MDS/MPN	32.9%	Haferlach T, et al. Leukemia 2013[55]
	CMML	6.0%	Patnaik MM, et al. Am J Hematol, 2013[51]
	CMML	6.0%	Itzykson R, et al. JCO, 2013[86]
	RARS-T	84.8%	Broséus J, et al. Leukemia, 2013[43]
	MPN	2.0%	Nangalia J, et al. NEJM, 2013[27]
	PMF	6.5%	Lasho TL, et al. Leukemia, 2011[87]
	Secondary AML derived from MPNs	3.8%	Zhang SJ, et al. Blood, 2012[39]
	de novo AML	0.5%	TCGA. NEJM, 2013[26]
	CLL	15.0%	Wang L, et al. NEJM, 2011[31]
	CLL	9.7%	Quesada V, et al. Nat Gent, 2012[32]
	Fludarabine-refractory CLL	17.0%	Rossi D, et al. Blood, 2011[33]

	Breast cancer	4.0%	Stephens PJ, et al. Nature, 2012[59]
	Breast cancer	3.9%	Ellis ML, et al. Nature, 2012[60]
	Pancreatic ductal adenocarcinoma	4.0%	ICGC. Nature, 2012[61]
	Uveral melanoma	19.0%	Harbour J, et al. Nat Gent, 2012[63]
U2AF1	MDS, CMML, AML	11.6%(MDS without RS), 8%(CMML), 9.7%(AML/MDS), 1.3%(AML)	Yoshida K, et al. Nature, 2011[22]
	MDS, CMML	8.7%	Graubert T, et al. Nat Gent, 2011[24]
	MDS, CMML, AML	6%(Low-risk MDS), 11%(High-risk MDS), 17%(CMML), 9%(AML)	Makishima H, et al. Blood, 2012[35]
	Lower-risk MDS	16.0%	Bejar R, et al. JCO, 2012[83]
	MDS	5.4%	Damm F, et al. Blood, 2012[45]
	MDS	7.3%	Thol F, et al. Blood, 2012[54]
	MDS, secondary AML	10.2%	Walter MJ, et al. Leukemia, 2013[84]
	MDS	10.0%	Mian SA, et al. Haematologica, 2013[85]
	MDS, MDS/MPN	6.1%	Papaemmanuil E, et al. Blood, 2013[56]
	MDS, MDS/MPN	7.7%	Haferlach T, et al. Leukemia 2013[55]
	CMML	9.0%	Patnaik MM, et al. Am J Hematol, 2013[51]
	CMML	5.0%	Itzykson R, et al. JCO, 2013[86]
	MPN	2.6%	Nangalia J, et al. NEJM, 2013[27]
	Therapy-related MDS and AML	2.6%	Shih AH, et al. Haematologica, 2013[88]
	Secondary AML derived from MPNs	5.7%	Zhang SJ, et al. Blood, 2012[39]
	de novo AML	4.0%	TCGA. NEJM, 2013[26]
	Advanced systemic mastocytosis	5.6%	Schwaab et al. blood, 2013[40]
	Childhood MDS, JMML	0.3%	Hirabayashi S, et al. Blood, 2012[28]
	JMML	3.7%	Takita J, et al. Leukemia, 2012[29]
	Lung adenocarcinoma	3.0%	Imielinski M, et al. Cell, 2012[65]

SRSF2	MDS, CMML, MPN	28.4%(CMML), 11.6%(MDS without RS), 5.5%(RARS, RCMD-RS), 6.5%(AML/MDS), 0.7%(AML), 1.9%(MPN)	Yoshida K, et al. Nature, 2011[22]
	MDS, CMML	28%(CMML)	Makishima H, et al. Blood, 2012[35]
	Lower-risk MDS	13.0%	Bejar R, et al. JCO, 2012[83]
	MDS	11.1%	Damm F, et al. Blood, 2012[45]
	MDS	12.4%	Thol F, et al. Blood, 2012[54]
	MDS	14.6%	Wu SJ, et al. Blood, 2012[49]
	MDS	13.0%	Mian SA, et al. Haematologica, 2013[85]
	MDS, MDS/MPN	14%	Papaemmanuil E, et al. Blood, 2013[56]
	MDS, MDS/MPN	17.5%	Haferlach T, et al. Leukemia 2013[55]
	CMML	47.0%	Meqqendorfer M, et al. Blood, 2012[50]
	CMML	40.0%	Patnaik MM, et al. Am J Hematol, 2013[51]
	MPN	1.3%	Nangalia J, et al. NEJM, 2013[27]
	PMF	17.0%	Lasho TL, et al. Blood, 2012[52]
	PMF	8.5%	Vannucchi AM, et al. Leukemia 2013[53]
	Secondary AML derived from MPNs	18.9%	Zhang SJ, et al. Blood, 2012[39]
	Therapy-related MDS and AML	7.9%	Shih AH, et al. Haematologica, 2013[88]
	de novo AML	0.5%	TCGA. NEJM, 2013[26]
	Advanced systemic mastocytosis	35.9%	Schwaab J, et al. blood, 2013[40]
	Childhood MDS, JMML	0.3%	Hirabayashi S, et al. Blood, 2012[28]
	JMML	3.7%	Takita J, et al. Leukemia, 2012[29]
ZRSR2	MDS, CMML	8%(CMML), 7.7%(MDS without RS), 1.4%(RARS, RCMD-RS), 1.6%(AML/MDS), 1.9%(MPN)	Yoshida K, et al. Nature, 2011[22]
	MDS, CMML	0.8%	Makishima H, et al. Blood, 2012[35]
	MDS	11.1%	Damm F, et al. Blood, 2012[45]
	MDS	3.1%	Thol F, et al. Blood, 2012[54]

	MDS, secondary AML	2.5%	Walter MJ, et al. Leukemia, 2013[84]
	MDS	1.0%	Mian SA, et al. Haematologica, 2013[85]
	MDS, MDS/MPN	4.5%	Papaemmanuil E, et al. Blood, 2013[56]
	MDS, MDS/MPN	7.6%	Haferlach T, et al. Leukemia 2013[55]
	CMML	8.0%	Itzykson R, et al. JCO, 2013[86]
	Secondary AML derived from MPNs	1.9%	Zhang SJ, et al. Blood, 2012[39]
SF3A1	MDS, CMML, AML	1.2%	Yoshida K, et al. Nature, 2011[22]
PRPF40B	MDS, CMML, AML	1.2%	Yoshida K, et al. Nature, 2011[22]
U2AF2	MDS, CMML, AML	0.7%	Yoshida K, et al. Nature, 2011[22]
	de novo AML	0.5%	TCGA. NEJM, 2013[26]
	CLL	1.9%	Quesada V, et al. Nat Gent, 2012[32]
SF1	MDS, CMML, AML	0.9%	Yoshida K, et al. Nature, 2011[22]
LUC7L2	MDS, CMML	0.8%	Makishima H, et al. Blood, 2012[35]
	MDS, secondary AML	0.6%	Walter MJ, et al. Leukemia, 2013[84]
PRPF8	MDS, CMML	0.8%	Makishima H, et al. Blood, 2012[35]
	de novo AML	2.0%	TCGA. NEJM, 2013[26]
SNRNP200	MDS, secondary AML	0.6%	Walter MJ, et al. Leukemia, 2013[84]
	de novo AML	0.5%	TCGA. NEJM, 2013[26]
CSTF2T	de novo AML	0.5%	TCGA. NEJM, 2013[26]
DDX1	de novo AML	0.5%	TCGA. NEJM, 2013[26]
DDX23	de novo AML	0.5%	TCGA. NEJM, 2013[26]
DHX32	de novo AML	0.5%	TCGA. NEJM, 2013[26]
HNRNPK	de novo AML	0.5%	TCGA. NEJM, 2013[26]
METTL3	de novo AML	0.5%	TCGA. NEJM, 2013[26]
PLRG1	de novo AML	0.5%	TCGA. NEJM, 2013[26]
POLR2A	de novo AML	0.5%	TCGA. NEJM, 2013[26]
PRPF3	de novo AML	0.5%	TCGA. NEJM, 2013[26]
RBMX	de novo AML	0.5%	TCGA. NEJM, 2013[26]
SRRM2	de novo AML	0.5%	TCGA. NEJM, 2013[26]
SRSF2	de novo AML	0.5%	TCGA. NEJM, 2013[26]
SRSF6	de novo AML	0.5%	TCGA. NEJM, 2013[26]
SUPT5H	de novo AML	0.5%	TCGA. NEJM, 2013[26]
TRA2B	de novo AML	0.5%	TCGA. NEJM, 2013[26]

U2AF1L4	de novo AML	0.5%	TCGA. NEJM, 2013[26]
CELF4	CLL	1.9%	Quesada V, et al. Nat Gent, 2012[32]
SFRS1	CLL	1.9%	Quesada V, et al. Nat Gent, 2012[32]
SFRS7	CLL	1.0%	Quesada V, et al. Nat Gent, 2012[32]

Abbreviations: JMML, juvenile myelomonocytic leukemia.

Figure Legends

Figure 1
Pre-mRNA and major spliceosomes

The U1, U2, U4/U6, U5 snRNPs are the main components of the major spliceosomes and each snRNP consists of a small nuclear RNA (snRNA) and a varying number of associated proteins. Assembly of spliceosomes begins with binding of U1 snRNP to the 5' splice site of intron. Subsequently, U2 snRNP interacts with the BPS (A complex) and then U4/U6.U5 tri-snRNP are recruited, forming the B complex. After the release of U1 and U4 snRNP, activated B complex undergo the catalytic process of splicing (C complex). Catalytic process of pre-mRNA splicing is a two-step process, during which splicesomes are assembled in a stepwise fashion as splicing proceeds.[1, 2] In the first step, the 5' splice site is cleaved and the 5' residue of the intron is linked to near the 3' splice junction. In the second step, the 3' splice site is cleaved, producing ligated exons and excised "lariat" introns. mRNA is released in the form of mRNA and associated protein (mRNP).

Figure 2
Frequent splicing factor mutations in myelodysplasia

Most of the mutations affected the components that are involved in the initial steps of pre-mRNA splicing including 5'-splice site recognition (*LUC7L2*) and 3'-splice site recognition (*SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*, *U2AF2, SF3A1* and *SF1*) (upper panel). These mutations are almost mutually exclusive, suggesting a common impact of these mutations on RNA splicing in the pathogenesis of myelodysplasia. Distribution of mutations in 8 spliceosome genes, where diagnosis of each sample is shown by indicated colors (lower panel).
W: Trp residues, RRM: RNA-recognition motif, UHM: U2AF homology motif domain, RS domain: arginine and serine rich domain

Figure 3
Reported mutations of splicing factor genes including *U2AF1*, *SRSF2*, *ZRSR2* and *SF3B1* in myelodysplasia

Mutations of *U2AF1*, *SRSF2* and *SF3B1* clustered in several hot spots (S34 and Q157 of *U2AF1*; P95 of *SRSF2*; E622, R625, H662, K666 and K700 of *SF3B1*), suggesting the gain-of-function nature of these mutations. On the other hand, *ZRSR2* mutations were widely distributed along the entire coding region and most of them were nonsense mutations or frameshift changes, or involved splicing donor/acceptor sites that caused either a premature truncation or a large structural change of the protein, leading to loss-of-function.
Zn: Zinc finger domain, UHM: U2AF homology motif domain, RRM: RNA recognition motif domain, HD: HEAT domain

Figure 4
A variety of splicing alterations observed in mutant U2AF1-transduced HeLa cells

U2AF1 S34F mutant allele induces a variety of abnormal RNA splicing in a global manner, including exon skipping, unspliced intron, altered splice site recognition and altered exon usage.

Figure 5
Potential of splicing factor mutations as therapeutic targets (synthetic lethality)

Cancer cells carrying splicing factor mutations (lower panel) are likely to have compromised pre-mRNA splicing and could be more sensitive to spliceosome inhibitors than unmutated cells (upper panel).

Fig 1

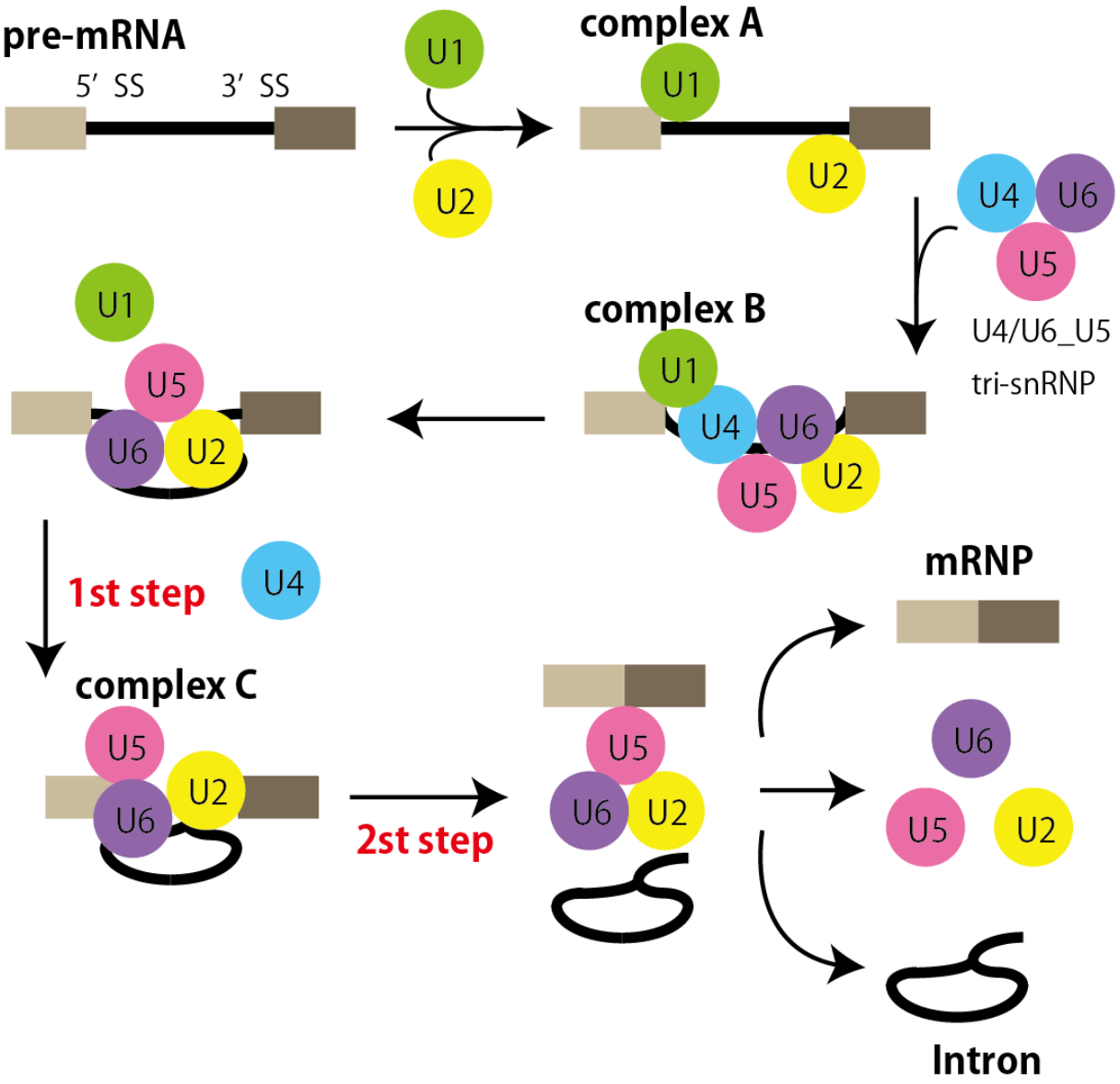
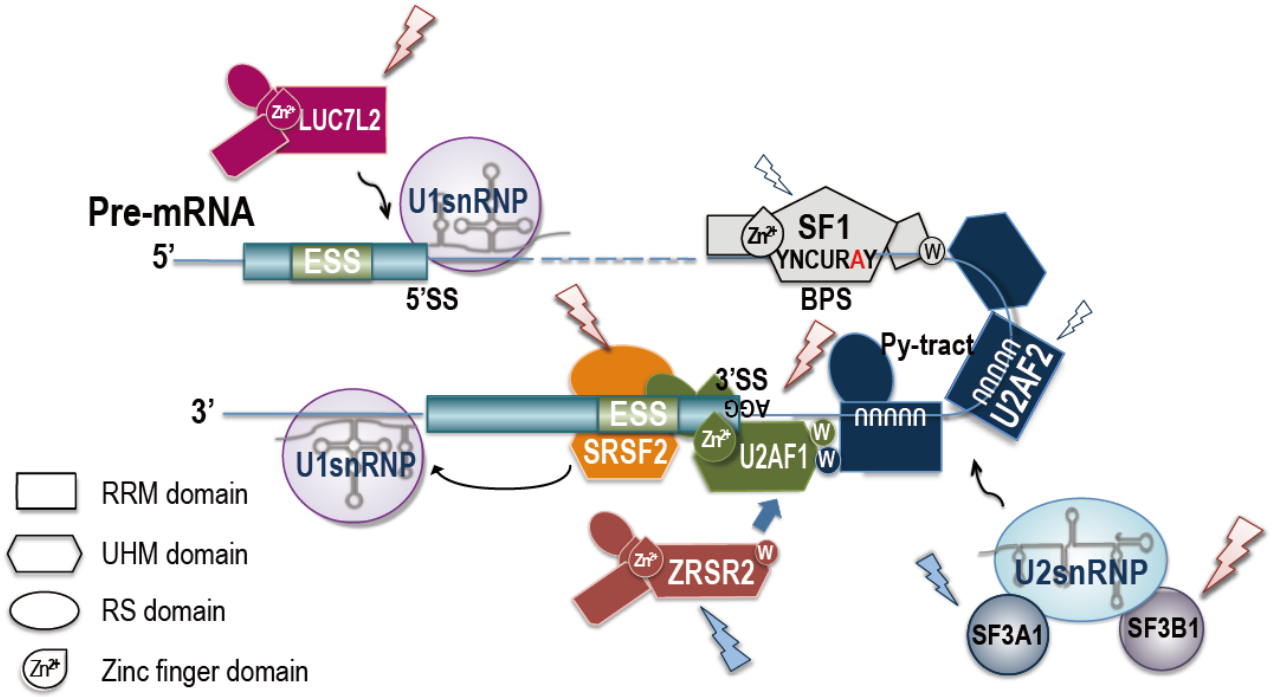


Fig 2



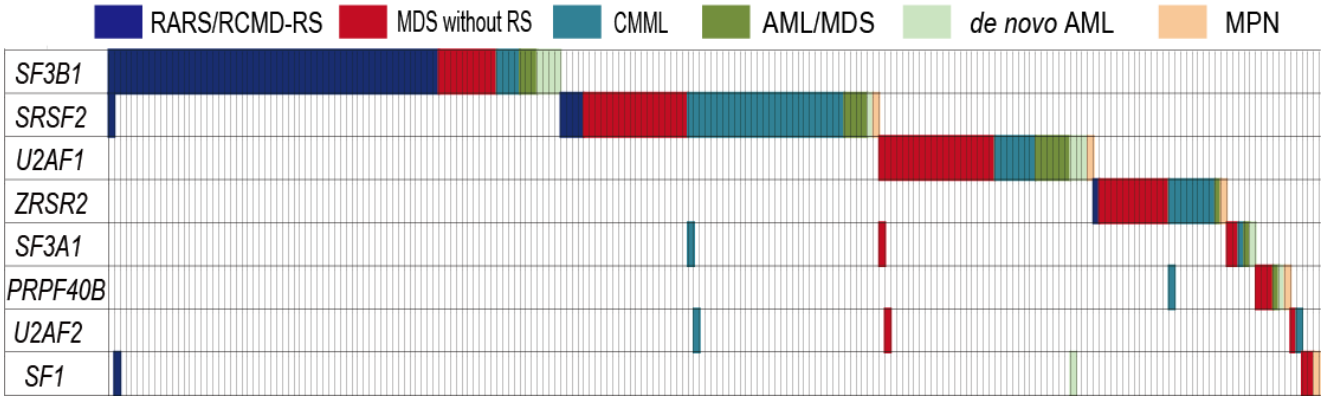


Fig 3

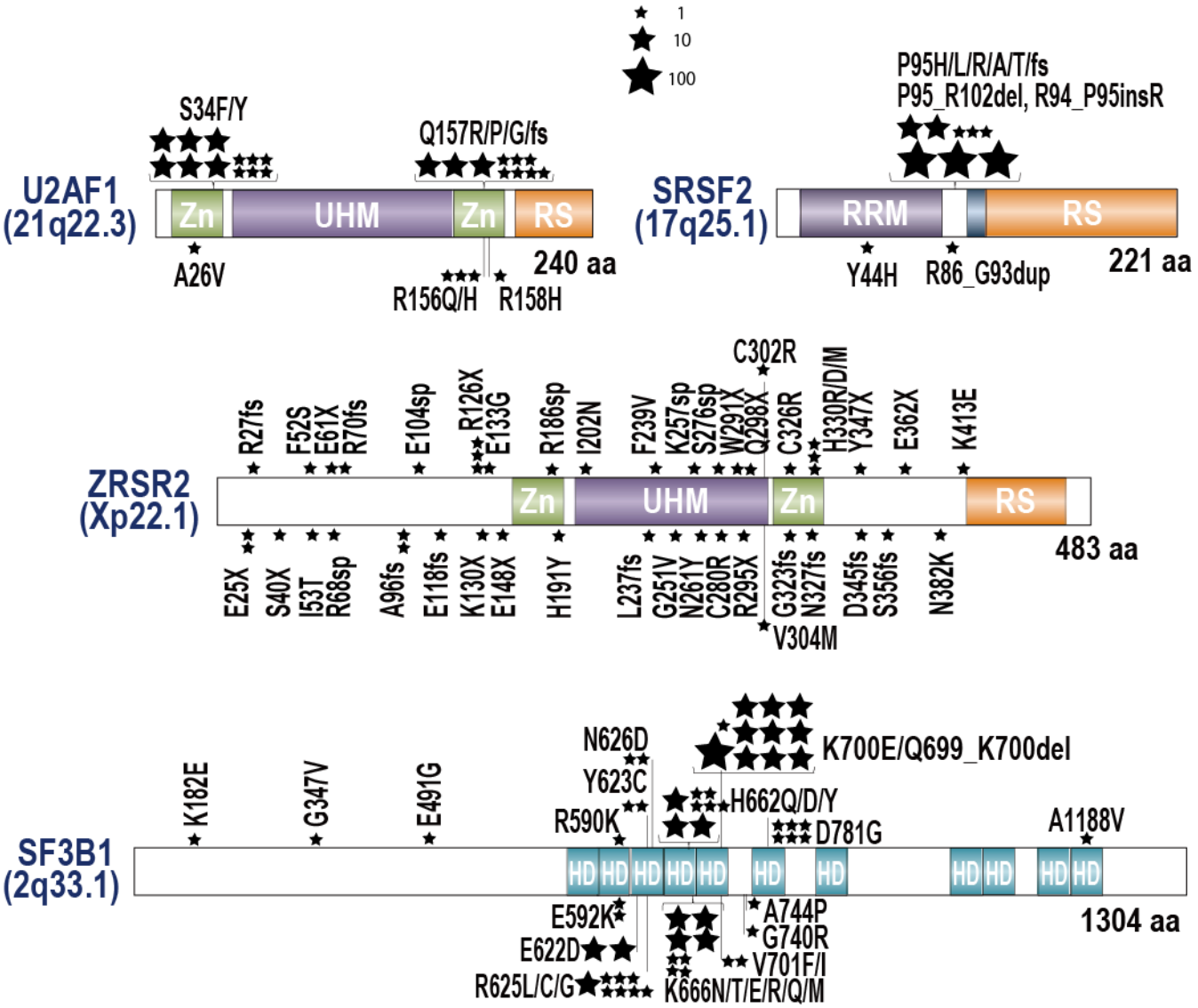


Fig 4

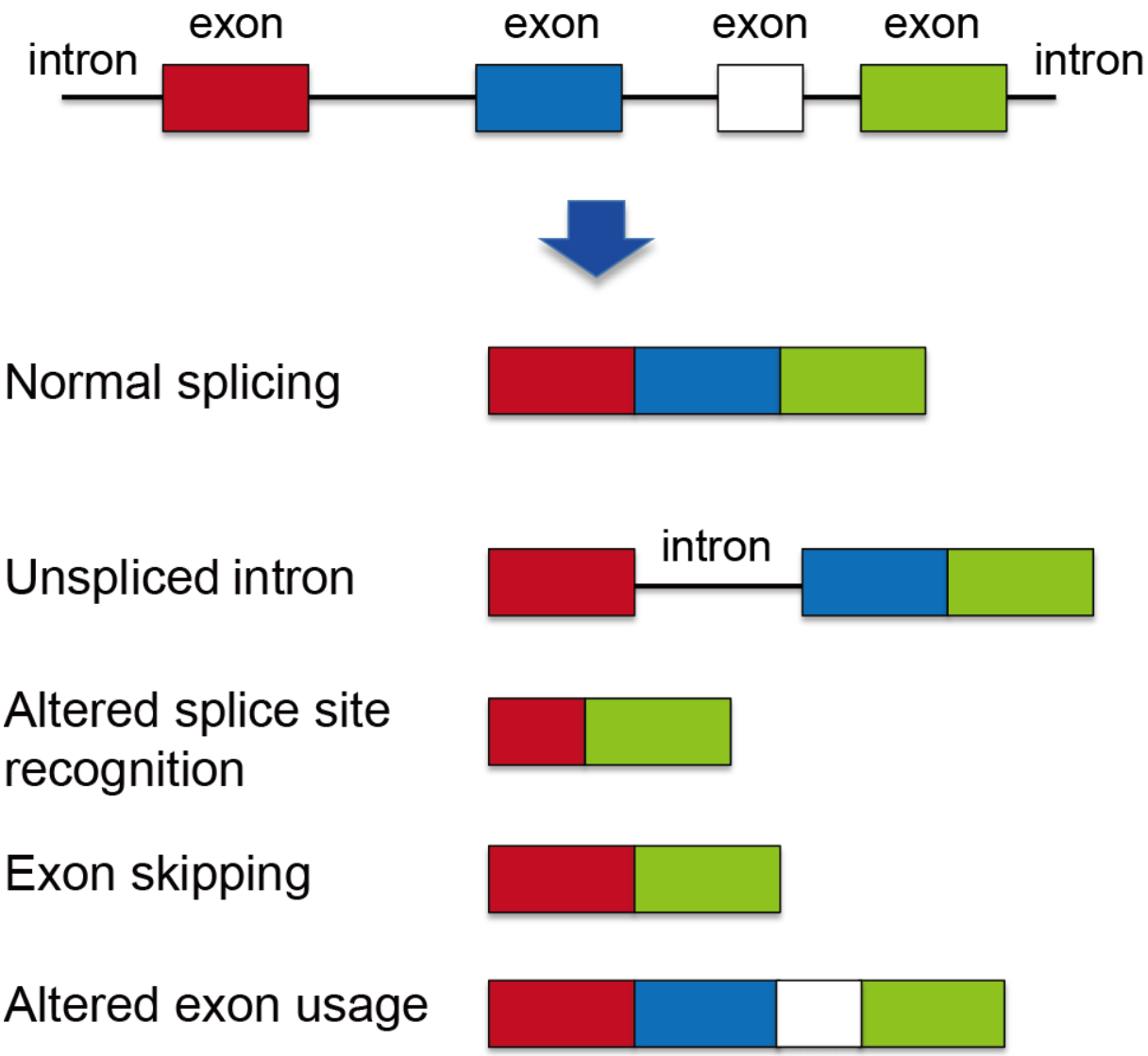
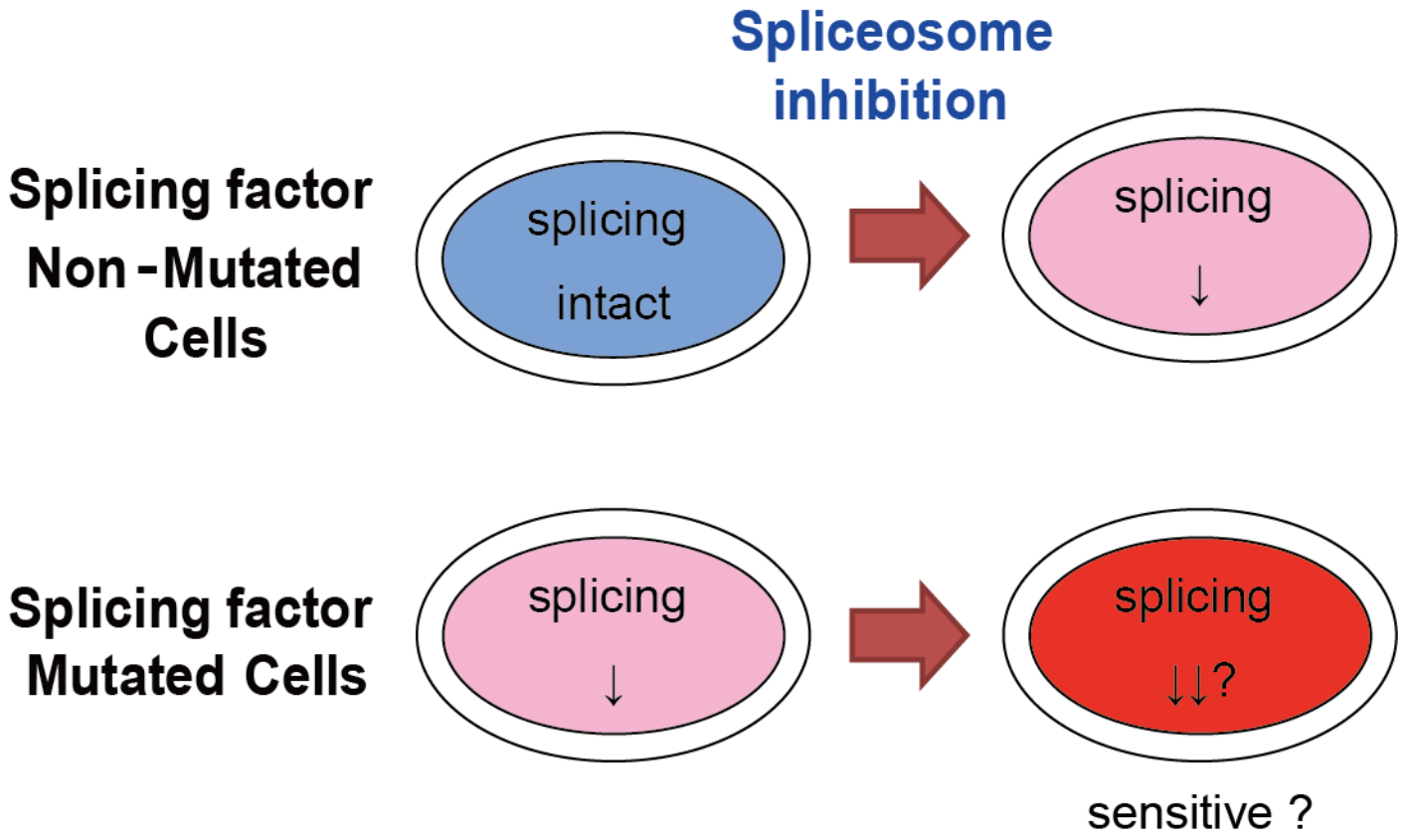


Fig 5



References

1. Wahl MC, Will CL, Luhrmann R: **The spliceosome: design principles of a dynamic RNP machine.** *Cell* 2009, **136:**701-718.
2. Kornblihtt AR, Schor IE, Allo M, Dujardin G, Petrillo E, Munoz MJ: **Alternative splicing: a pivotal step between eukaryotic transcription and translation.** *Nat Rev Mol Cell Biol* 2013, **14:**153-165.
3. Turunen JJ, Niemela EH, Verma B, Frilander MJ: **The significant other: splicing by the minor spliceosome.** *Wiley Interdiscip Rev RNA* 2013, **4:**61-76.
4. Lin S, Coutinho-Mansfield G, Wang D, Pandit S, Fu XD: **The splicing factor SC35 has an active role in transcriptional elongation.** *Nat Struct Mol Biol* 2008, **15:**819-826.
5. Maciejewski JP, Padgett RA: **Defects in spliceosomal machinery: a new pathway of leukaemogenesis.** *Br J Haematol* 2012, **158:**165-173.
6. Cazzola M, Rossi M, Malcovati L: **Biologic and clinical significance of somatic mutations of SF3B1 in myeloid and lymphoid neoplasms.** *Blood* 2013, **121:**260-269.
7. Wan Y, Wu CJ: **SF3B1 mutations in chronic lymphocytic leukemia.** *Blood* 2013, **121:**4627-4634.
8. Wang GS, Cooper TA: **Splicing in disease: disruption of the splicing code and the decoding machinery.** *Nat Rev Genet* 2007, **8:**749-761.
9. Cartegni L, Chew SL, Krainer AR: **Listening to silence and understanding nonsense: exonic mutations that affect splicing.** *Nat Rev Genet* 2002, **3:**285-298.
10. Cooper TA, Wan L, Dreyfuss G: **RNA and disease.** *Cell* 2009, **136:**777-793.
11. Bernier FP, Caluseriu O, Ng S, Schwartzentruber J, Buckingham KJ, Innes AM, Jabs EW, Innis JW, Schuette JL, Gorski JL, et al: **Haploinsufficiency of SF3B4, a component of the pre-mRNA spliceosomal complex, causes Nager syndrome.** *Am J Hum Genet* 2012, **90:**925-933.
12. Edery P, Marcaillou C, Sahbatou M, Labalme A, Chastang J, Touraine R, Tubacher E, Senni F, Bober MB, Nampoothiri S, et al: **Association of TALS developmental disorder with defect in minor splicing component U4atac snRNA.** *Science* 2011, **332:**240-243.
13. He H, Liyanarachchi S, Akagi K, Nagy R, Li J, Dietrich RC, Li W, Sebastian N, Wen B, Xin B, et al: **Mutations in U4atac snRNA, a component of the minor spliceosome, in the developmental disorder MOPD I.** *Science* 2011, **332:**238-240.
14. Mordes D, Yuan L, Xu L, Kawada M, Molday RS, Wu JY: **Identification of photoreceptor genes affected by PRPF31 mutations associated with autosomal dominant retinitis pigmentosa.** *Neurobiol Dis* 2007, **26:**291-300.
15. David CJ, Manley JL: **Alternative pre-mRNA splicing regulation in cancer: pathways and programs unhinged.** *Genes Dev* 2010, **24:**2343-2364.
16. Kim E, Goren A, Ast G: **Insights into the connection between cancer and alternative splicing.** *Trends Genet* 2008, **24:**7-10.
17. Xerri L, Hassoun J, Devilard E, Birnbaum D, Birg F: **BCL-X and the apoptotic machinery of lymphoma cells.** *Leuk Lymphoma* 1998, **28:**451-458.
18. Fushimi K, Ray P, Kar A, Wang L, Sutherland LC, Wu JY: **Up-regulation of the proapoptotic caspase 2 splicing isoform by a candidate tumor suppressor, RBM5.** *Proc Natl Acad Sci U S A* 2008, **105:**15708-15713.
19. Sheen-Chen SM, Chen HS, Eng HL, Chen WJ: **Circulating soluble Fas in patients with breast cancer.** *World J Surg* 2003, **27:**10-13.
20. Karni R, de Stanchina E, Lowe SW, Sinha R, Mu D, Krainer AR: **The gene encoding the splicing factor SF2/ASF is a proto-oncogene.** *Nat Struct Mol Biol* 2007, **14:**185-193.
21. Tefferi A, Vardiman JW: **Myelodysplastic syndromes.** *N Engl J Med* 2009, **361:**1872-1885.

22. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, Sato Y, Sato-Otsubo A, Kon A, Nagasaki M, et al: **Frequent pathway mutations of splicing machinery in myelodysplasia.** *Nature* 2011, **478:**64-69.
23. Papaemmanuil E, Cazzola M, Boultwood J, Malcovati L, Vyas P, Bowen D, Pellagatti A, Wainscoat JS, Hellstrom-Lindberg E, Gambacorti-Passerini C, et al: **Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts.** *N Engl J Med* 2011, **365:**1384-1395.
24. Graubert TA, Shen D, Ding L, Okeyo-Owuor T, Lunn CL, Shao J, Krysiak K, Harris CC, Koboldt DC, Larson DE, et al: **Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes.** *Nat Genet* 2011, **44:**53-57.
25. Visconte V, Makishima H, Jankowska A, Szpurka H, Traina F, Jerez A, O'Keefe C, Rogers HJ, Sekeres MA, Maciejewski JP, Tiu RV: **SF3B1, a splicing factor is frequently mutated in refractory anemia with ring sideroblasts.** *Leukemia* 2011, **26:**542-545.
26. TCGA: **Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia.** *N Engl J Med* 2013, **368:**2059-2074.
27. Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC, Avezov E, Li J, Kollmann K, Kent DG, et al: **Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2.** *N Engl J Med* 2013, **369:**2391-2405.
28. Hirabayashi S, Flotho C, Moetter J, Heuser M, Hasle H, Gruhn B, Klingebiel T, Thol F, Schlegelberger B, Baumann I, et al: **Spliceosomal gene aberrations are rare, coexist with oncogenic mutations, and are unlikely to exert a driver effect in childhood MDS and JMML.** *Blood* 2012, **119:**e96-99.
29. Takita J, Yoshida K, Sanada M, Nishimura R, Okubo J, Motomura A, Hiwatari M, Oki K, Igarashi T, Hayashi Y, Ogawa S: **Novel splicing-factor mutations in juvenile myelomonocytic leukemia.** *Leukemia* 2012, **26:**1879-1881.
30. Je EM, Yoo NJ, Kim YJ, Kim MS, Lee SH: **Mutational analysis of splicing machinery genes SF3B1, U2AF1 and SRSF2 in myelodysplasia and other common tumors.** *Int J Cancer* 2013, **133:**260-265.
31. Wang L, Lawrence MS, Wan Y, Stojanov P, Sougnez C, Stevenson K, Werner L, Sivachenko A, DeLuca DS, Zhang L, et al: **SF3B1 and other novel cancer genes in chronic lymphocytic leukemia.** *N Engl J Med* 2011, **365:**2497-2506.
32. Quesada V, Conde L, Villamor N, Ordonez GR, Jares P, Bassaganyas L, Ramsay AJ, Bea S, Pinyol M, Martinez-Trillos A, et al: **Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia.** *Nat Genet* 2011, **44:**47-52.
33. Rossi D, Bruscaggin A, Spina V, Rasi S, Khiabanian H, Messina M, Fangazio M, Vaisitti T, Monti S, Chiaretti S, et al: **Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness.** *Blood* 2011, **118:**6904-6908.
34. Ramsay AJ, Rodriguez D, Villamor N, Kwarciak A, Tejedor JR, Valcarcel J, Lopez-Guillermo A, Martinez-Trillos A, Puente XS, Campo E, et al: **Frequent somatic mutations in components of the RNA processing machinery in chronic lymphocytic leukemia.** *Leukemia* 2013, **27:**1600-1603.
35. Makishima H, Visconte V, Sakaguchi H, Jankowska AM, Abu Kar S, Jerez A, Przychodzen B, Bupathi M, Guinta K, Afable MG, et al: **Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis.** *Blood* 2012, **119:**3203-3210.
36. Landau DA, Carter SL, Stojanov P, McKenna A, Stevenson K, Lawrence MS, Sougnez C, Stewart C, Sivachenko A, Wang L, et al: **Evolution and impact of subclonal mutations in chronic lymphocytic leukemia.** *Cell* 2013, **152:**714-726.

37. Waterfall JJ, Arons E, Walker RL, Pineda M, Roth L, Killian JK, Abaan OD, Davis SR, Kreitman RJ, Meltzer PS: **High prevalence of MAP2K1 mutations in variant and IGHV4-34-expressing hairy-cell leukemias.** *Nat Genet* 2014, **46:**8-10.
38. Chen M, Manley JL: **Mechanisms of alternative splicing regulation: insights from molecular and genomics approaches.** *Nat Rev Mol Cell Biol* 2009, **10:**741-754.
39. Zhang SJ, Rampal R, Manshouri T, Patel J, Mensah N, Kayserian A, Hricik T, Heguy A, Hedvat C, Gonen M, et al: **Genetic analysis of patients with leukemic transformation of myeloproliferative neoplasms shows recurrent SRSF2 mutations that are associated with adverse outcome.** *Blood* 2012, **119:**4480-4485.
40. Schwaab J, Schnittger S, Sotlar K, Walz C, Fabarius A, Pfirrmann M, Kohlmann A, Grossmann V, Meggendorfer M, Horny HP, et al: **Comprehensive mutational profiling in advanced systemic mastocytosis.** *Blood* 2013, **122:**2460-2466.
41. Malcovati L, Papaemmanuil E, Bowen DT, Boultwood J, Della Porta MG, Pascutto C, Travaglino E, Groves MJ, Godfrey AL, Ambaglio I, et al: **Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms.** *Blood* 2011, **118:**6239-6246.
42. Patnaik MM, Lasho TL, Hodnefield JM, Knudson RA, Ketterling RP, Garcia-Manero G, Steensma DP, Pardanani A, Hanson CA, Tefferi A: **SF3B1 mutations are prevalent in myelodysplastic syndromes with ring sideroblasts but do not hold independent prognostic value.** *Blood* 2011, **119:**569-572.
43. Broseus J, Alpermann T, Wulfert M, Florensa Brichs L, Jeromin S, Lippert E, Rozman M, Lifermann F, Grossmann V, Haferlach T, et al: **Age, JAK2 and SF3B1 mutations are the main predicting factors for survival in refractory anaemia with ring sideroblasts and marked thrombocytosis.** *Leukemia* 2013, **27:**1826-1831.
44. Shen H, Zheng X, Luecke S, Green MR: **The U2AF35-related protein Urp contacts the 3' splice site to promote U12-type intron splicing and the second step of U2-type intron splicing.** *Genes Dev* 2010, **24:**2389-2394.
45. Damm F, Kosmider O, Gelsi-Boyer V, Renneville A, Carbuccia N, Hidalgo-Curtis C, Della Valle V, Couronne L, Scourzic L, Chesnais V, et al: **Mutations affecting mRNA splicing define distinct clinical phenotypes and correlate with patient outcome in myelodysplastic syndromes.** *Blood* 2012, **119:**3211-3218.
46. Singh H, Lane AA, Correll M, Przychodzen B, Sykes DB, Stone RM, Ballen KK, Amrein PC, Maciejewski J, Attar EC: **Putative RNA-splicing gene LUC7L2 on 7q34 represents a candidate gene in pathogenesis of myeloid malignancies.** *Blood Cancer J* 2013, **3:**e117.
47. Keightley MC, Crowhurst MO, Layton JE, Beilharz T, Markmiller S, Varma S, Hogan BM, de Jong-Curtain TA, Heath JK, Lieschke GJ: **In vivo mutation of pre-mRNA processing factor 8 (Prpf8) affects transcript splicing, cell survival and myeloid differentiation.** *FEBS Lett* 2013, **587:**2150-2157.
48. Damm F, Thol F, Kosmider O, Kade S, Loffeld P, Dreyfus F, Stamatoullas-Bastard A, Tanguy-Schmidt A, Beyne-Rauzy O, de Botton S, et al: **SF3B1 mutations in myelodysplastic syndromes: clinical associations and prognostic implications.** *Leukemia* 2011, **26:**1137-1140.
49. Wu SJ, Kuo YY, Hou HA, Li LY, Tseng MH, Huang CF, Lee FY, Liu MC, Liu CW, Lin CT, et al: **The clinical implication of SRSF2 mutation in patients with myelodysplastic syndrome and its stability during disease evolution.** *Blood* 2012, **120:**3106-3111.
50. Meggendorfer M, Roller A, Haferlach T, Eder C, Dicker F, Grossmann V, Kohlmann A, Alpermann T, Yoshida K, Ogawa S, et al: **SRSF2 mutations in 275 cases with chronic myelomonocytic leukemia (CMML).** *Blood* 2012, **120:**3080-3088.
51. Patnaik MM, Lasho TL, Finke CM, Hanson CA, Hodnefield JM, Knudson RA, Ketterling RP, Pardanani A, Tefferi A: **Spliceosome mutations involving SRSF2, SF3B1, and**

U2AF35 in chronic myelomonocytic leukemia: prevalence, clinical correlates, and prognostic relevance. *Am J Hematol* 2013, **88:**201-206.

52. Lasho TL, Jimma T, Finke CM, Patnaik M, Hanson CA, Ketterling RP, Pardanani A, Tefferi A: **SRSF2 mutations in primary myelofibrosis: significant clustering with IDH mutations and independent association with inferior overall and leukemia-free survival.** *Blood* 2012, **120:**4168-4171.
53. Vannucchi AM, Lasho TL, Guglielmelli P, Biamonte F, Pardanani A, Pereira A, Finke C, Score J, Gangat N, Mannarelli C, et al: **Mutations and prognosis in primary myelofibrosis.** *Leukemia* 2013, **27:**1861-1869.
54. Thol F, Kade S, Schlarmann C, Loffeld P, Morgan M, Krauter J, Wlodarski MW, Kolking B, Wichmann M, Gorlich K, et al: **Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes.** *Blood* 2012, **119:**3578-3584.
55. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, Schnittger S, Sanada M, Kon A, Alpermann T, et al: **Landscape of Genetic Lesions in 944 Patients with Myelodysplastic Syndromes.** *Leukemia* 2013 [epub ahead of print].
56. Papaemmanuil E, Gerstung M, Malcovati L, Tauro S, Gundem G, Van Loo P, Yoon CJ, Ellis P, Wedge DC, Pellagatti A, et al: **Clinical and biological implications of driver mutations in myelodysplastic syndromes.** *Blood* 2013, **122:**3616-3627.
57. Oscier DG, Rose-Zerilli MJ, Winkelmann N, Gonzalez de Castro D, Gomez B, Forster J, Parker H, Parker A, Gardiner A, Collins A, et al: **The clinical significance of NOTCH1 and SF3B1 mutations in the UK LRF CLL4 trial.** *Blood* 2013, **121:**468-475.
58. Rossi D, Rasi S, Spina V, Bruscaggin A, Monti S, Ciardullo C, Deambrogi C, Khiabanian H, Serra R, Bertoni F, et al: **Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia.** *Blood* 2013, **121:**1403-1412.
59. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, Nik-Zainal S, Martin S, Varela I, Bignell GR, et al: **The landscape of cancer genes and mutational processes in breast cancer.** *Nature* 2012, **486:**400-404.
60. Ellis MJ, Ding L, Shen D, Luo J, Suman VJ, Wallis JW, Van Tine BA, Hoog J, Goiffon RJ, Goldstein TC, et al: **Whole-genome analysis informs breast cancer response to aromatase inhibition.** *Nature* 2012, **486:**353-360.
61. Biankin AV, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL, Miller DK, Wilson PJ, Patch AM, Wu J, et al: **Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes.** *Nature* 2012, **491:**399-405.
62. Stephens PJ, Davies HR, Mitani Y, Van Loo P, Shlien A, Tarpey PS, Papaemmanuil E, Cheverton A, Bignell GR, Butler AP, et al: **Whole exome sequencing of adenoid cystic carcinoma.** *J Clin Invest* 2013, **123:**2965-2968.
63. Harbour JW, Roberson ED, Anbunathan H, Onken MD, Worley LA, Bowcock AM: **Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma.** *Nat Genet* 2013, **45:**133-135.
64. Martin M, Masshofer L, Temming P, Rahmann S, Metz C, Bornfeld N, van de Nes J, Klein-Hitpass L, Hinnebusch AG, Horsthemke B, et al: **Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3.** *Nat Genet* 2013, **45:**933-936.
65. Imielinski M, Berger AH, Hammerman PS, Hernandez B, Pugh TJ, Hodis E, Cho J, Suh J, Capelletti M, Sivachenko A, et al: **Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing.** *Cell* 2012, **150:**1107-1120.
66. Itzykson R, Kosmider O, Renneville A, Morabito M, Preudhomme C, Berthon C, Ades L, Fenaux P, Platzbecker U, Gagey O, et al: **Clonal architecture of chronic myelomonocytic leukemias.** *Blood* 2013, **121:**2186-2198.

67. Jeromin S, Haferlach T, Grossmann V, Alpermann T, Kowarsch A, Haferlach C, Kern W, Schnittger S: **High frequencies of SF3B1 and JAK2 mutations in refractory anemia with ring sideroblasts associated with marked thrombocytosis strengthen the assignment to the category of myelodysplastic/myeloproliferative neoplasms.** *Haematologica* 2013, **98:**e15-17.
68. McDuff FK, Turner SD: **Jailbreak: oncogene-induced senescence and its evasion.** *Cell Signal* 2011, **23:**6-13.
69. Visconte V, Rogers HJ, Singh J, Barnard J, Bupathi M, Traina F, McMahon J, Makishima H, Szpurka H, Jankowska A, et al: **SF3B1 haploinsufficiency leads to formation of ring sideroblasts in myelodysplastic syndromes.** *Blood* 2012, **120:**3173-3186.
70. Guth S, Martinez C, Gaur RK, Valcarcel J: **Evidence for substrate-specific requirement of the splicing factor U2AF(35) and for its function after polypyrimidine tract recognition by U2AF(65).** *Mol Cell Biol* 1999, **19:**8263-8271.
71. Pacheco TR, Coelho MB, Desterro JM, Mollet I, Carmo-Fonseca M: **In vivo requirement of the small subunit of U2AF for recognition of a weak 3' splice site.** *Mol Cell Biol* 2006, **26:**8183-8190.
72. Przychodzen B, Jerez A, Guinta K, Sekeres MA, Padgett R, Maciejewski JP, Makishima H: **Patterns of missplicing due to somatic U2AF1 mutations in myeloid neoplasms.** *Blood* 2013, **122:**999-1006.
73. Furney SJ, Pedersen M, Gentien D, Dumont AG, Rapinat A, Desjardins L, Turajlic S, Piperno-Neumann S, de la Grange P, Roman-Roman S, et al: **SF3B1 mutations are associated with alternative splicing in uveal melanoma.** *Cancer Discov* 2013, **3:**1122-1129.
74. Allo M, Schor IE, Munoz MJ, de la Mata M, Agirre E, Valcarcel J, Eyras E, Kornblihtt AR: **Chromatin and alternative splicing.** *Cold Spring Harb Symp Quant Biol* 2010, **75:**103-111.
75. Luco RF, Allo M, Schor IE, Kornblihtt AR, Misteli T: **Epigenetics in alternative pre-mRNA splicing.** *Cell* 2011, **144:**16-26.
76. Isono K, Mizutani-Koseki Y, Komori T, Schmidt-Zachmann MS, Koseki H: **Mammalian polycomb-mediated repression of Hox genes requires the essential spliceosomal protein Sf3b1.** *Genes Dev* 2005, **19:**536-541.
77. Xiao R, Sun Y, Ding JH, Lin S, Rose DW, Rosenfeld MG, Fu XD, Li X: **Splicing regulator SC35 is essential for genomic stability and cell proliferation during mammalian organogenesis.** *Mol Cell Biol* 2007, **27:**5393-5402.
78. Shaheen M, Allen C, Nickoloff JA, Hromas R: **Synthetic lethality: exploiting the addiction of cancer to DNA repair.** *Blood* 2011, **117:**6074-6082.
79. Kaida D, Motoyoshi H, Tashiro E, Nojima T, Hagiwara M, Ishigami K, Watanabe H, Kitahara T, Yoshida T, Nakajima H, et al: **Spliceostatin A targets SF3b and inhibits both splicing and nuclear retention of pre-mRNA.** *Nat Chem Biol* 2007, **3:**576-583.
80. Kotake Y, Sagane K, Owa T, Mimori-Kiyosue Y, Shimizu H, Uesugi M, Ishihama Y, Iwata M, Mizui Y: **Splicing factor SF3b as a target of the antitumor natural product pladienolide.** *Nat Chem Biol* 2007, **3:**570-575.
81. Fan L, Lagisetti C, Edwards CC, Webb TR, Potter PM: **Sudemycins, novel small molecule analogues of FR901464, induce alternative gene splicing.** *ACS Chem Biol* 2011, **6:**582-589.
82. Albert BJ, McPherson PA, O'Brien K, Czaicki NL, Destefino V, Osman S, Li M, Day BW, Grabowski PJ, Moore MJ, et al: **Meayamycin inhibits pre-messenger RNA splicing and exhibits picomolar activity against multidrug-resistant cells.** *Mol Cancer Ther* 2009, **8:**2308-2318.
83. Bejar R, Stevenson KE, Caughey BA, Abdel-Wahab O, Steensma DP, Galili N, Raza A, Kantarjian H, Levine RL, Neuberg D, et al: **Validation of a prognostic model and the**

impact of mutations in patients with lower-risk myelodysplastic syndromes. *J Clin Oncol* 2012, **30:**3376-3382.

84. Walter MJ, Shen D, Shao J, Ding L, White BS, Kandoth C, Miller CA, Niu B, McLellan MD, Dees ND, et al: **Clonal diversity of recurrently mutated genes in myelodysplastic syndromes.** *Leukemia* 2013, **27:**1275-1282.
85. Mian SA, Smith AE, Kulasekararaj AG, Kizilors A, Mohamedali AM, Lea NC, Mitsopoulos K, Ford K, Nasser E, Seidl T, Mufti GJ: **Spliceosome mutations exhibit specific associations with epigenetic modifiers and proto-oncogenes mutated in myelodysplastic syndrome.** *Haematologica* 2013, **98:**1058-1066.
86. Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M, Berthon C, Ades L, Fenaux P, Beyne-Rauzy O, et al: **Prognostic score including gene mutations in chronic myelomonocytic leukemia.** *J Clin Oncol* 2013, **31:**2428-2436.
87. Lasho TL, Finke CM, Hanson CA, Jimma T, Knudson RA, Ketterling RP, Pardanani A, Tefferi A: **SF3B1 mutations in primary myelofibrosis: clinical, histopathology and genetic correlates among 155 patients.** *Leukemia* 2011, **26:**1135-1137.
88. Shih AH, Chung SS, Dolezal EK, Zhang SJ, Abdel-Wahab OI, Park CY, Nimer SD, Levine RL, Klimek VM: **Mutational analysis of therapy-related myelodysplastic syndromes and acute myelogenous leukemia.** *Haematologica* 2013, **98:**908-912.